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METHODS OF STUDYING FOREST ECOSYSTEMS: MICROORGANISM AND SAPROPHAGE CONSUMPTION IN THE LITTER*

ABSTRACT: The paper presents methods for the evaluation of litter consumption by microorganisms and saprophages under natural conditions without any interference from the investigator. As has been found, in an alder wood microorganisms consume a total of 498 kcal/m^2 (26.5% of total loss), and the saprophages 1,382 kcal/m^2 (73.5% of loss) a year. For the microorganisms the highest litter consumption rate was recorded for the period immediately following the falling of leaves, and for the saprophages during the spring-summer months.

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1. INTRODUCTION

One of the basic problems encountered in studying litter decomposition is the necessity to know the contribution of individual destruent groups. To find whether decomposition is caused to a greater extent by microorganisms, or by saprophages, a number of methods is used, as a matter of fact all are based on similar principles: the exclusion (or limiting the activity) of one of the litter-consumer groups studied. Exclusion from activity may be brought about by a mechanical factor such as the use of nets with a required mesh-size, or by a chemical factor, such as the application of toluene and naphthalene which exclude saprophages, or microorganisms (K u r č e v a 1960, C r o s s l e y and H o g l u n d 1962, E d w a r d s and

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Heath 1963, Witkamp and Crossley 1966, Zlotin 1970). An interesting approach to the problem of saprophage activity evaluation is the indirect assessment on the basis of litter-surface losses, because it is certain that changes in the quantity of litter are caused by the activity of saprophages and not by microorganisms (Heath, Edwards and Arnold 1964).

The aim of the present paper is to present methods of evaluating litter consumption by microorganisms and saprophages under natural conditions, without any experimental interference of the investigator. It partly represents a further development of the methods used by Heath, Edwards and Arnold (1964).

2. ASSUMPTIONS CONCERNING STUDY METHODS

Because most leaf-fall in the temperate-zone forest ecosystems occurs during the autumn months, it is relatively easy to follow the fate of leaves of a particular leaf fall, over a yearly cycle, for all one needs to do is to consistently identify leaves of the new falling cycle among the leaves accumulated in the litter. This is easy, because the leaves in question are of a different colour and degree of fragmentation, etc. Obviously, the yearly variations in the weight of the leaves of a particular litter fall will always show a more or less pronounced tendency to decrease, this being the result of the progressing decomposition processes caused by the combined action of microorganisms and saprophages.

In accordance with the assumptions of Heath, Edwards and Arnold (1964), saprophage consumption may be restricted to leaf-surface losses. Thus all one needs to do to express saprophage consumption in time $t_o - t_{+1}$ by the formula (1) given below is to specify the annual variations in weight of the leaves found over 1 m² per unit area (e.g., m^2/m^2).

$$C_{s_{t_o - t_{+1}}} = S_{t_o} - S_{t_{+1}} \quad \text{or} \quad C_s = \Delta S \tag{1}$$

where: S_{t_o} is the surface of leaves accumulated over 1 m² during time t_o, and $S_{t_{+1}}$ – the surface of leaves in the next point of time t_{+1}.

The most convenient way of transposing the weight of leaves from units of weight to surface units seems to be by the method suggested by Stachurski and Zimka (1975) which is used for the calculation of the assimilation surface of the foliage on the basis of the known weight of fallen leaves per 1 m². In this method, for the weight-surface conversion the following formula is used:

$$S = \frac{W}{wg} \tag{2}$$

where: W is the weight of falling leaves per 1 m² and wg – the weight of a foliage-surface unit (= 1 cm²) of the particular species[1]. This method is much more convenient than the calculation

[1] This quantity is obtained by cutting out discs of known size from randomly selected leaves, from parts not gnawn by animals.

of leaf surface by using planimetric, or photometric methods. With the progressing leaf fragmentation over the yearly cycle these methods become very laborious, which makes the elaboration of the material difficult.

The expression ΔS, which defines the saprophage consumption, is not, however, precise enough, because leaf surface weight (even of the same plant species) may vary considerably from one point of time to another. This objection may easily be eliminated by converting leaf-surface losses in m^2 into units that are fully comparable, i.e., units of weight. Leaf consumption by saprophages is then quantified as follows:

$$C_{S_{t_o - t_{+1}}} = S \frac{wg_{t_o} + wg_{t_{+1}}}{2} \quad \text{or} \quad C_s = \Delta S \cdot wg \tag{3}$$

where: ΔS is used exactly as above, while wg_{t_o} and $wg_{t_{+1}}$ signify the weight of 1 cm^2 leaf surface during time t_o and t_{+1}.

Saprophage consumption so defined may be quantified in terms of grams, kcal, or weight units of any element that we may be interested in. Obviously, the total annual saprophage consumption will be the sum total of consumption quantities in the consecutive points of time.

While litter consumption by saprophages causes a gradual decrease of its total surface, the activity of microorganisms may be defined as one resulting in weight decrease of the existing surface by reducing the thickness of the leaves. Microorganism consumption per 1 cm^2 leaves accumulated may, therefore, be expressed by the formula:

$$C_{m\ t_o - t_{+1}} = wg_{t_o} - wg_{t_{+1}} \quad \text{or} \quad C_m = \Delta wg \tag{4}$$

whereas the consumption caused by microorganisms per total surface of leaves accumulated over 1 m^2 is expressed by the formula:

$$C_{m\ t_o - t_{+1}} = \Delta wg \frac{S_{t_o} + S_{t_{+1}}}{2} \quad \text{or} \quad C_m = \Delta wg \bar{S} \tag{5}$$

The meaning of the symbols is the same as for the saprophages. As in the case of saprophages, the total annual microorganism consumption is obtained by summing the consumption quantities per consecutive points of time over the yearly cycle.

3. STUDY AREA AND METHODS

The study was carried out in an alder wood (*Carici elongatae-Alnetum*), 80 years old. This ecosystem consists of hollows periodically flooded, and of higher parts – islands which are never flooded. The investigations were carried out only in the elevated parts of the alder wood during the period November 1973-November 1974. In order to determine the decomposition rate of the leaves of the 1973 falling cycle, 2 types of litter sample were collected 6 times a year.

1. Samples characterising the weight of leaves of all species, accumulated over 1 m^2. These were obtained by cutting out soil cores, 10 cm^2 in section, at the depth of 5 cm. During each series 30 such samples were collected. On account of the relatively small sample area, the material provided by the samples was not sufficient, for instance, for chemical analyses. For this reason, samples of another type were collected simultaneously.

2. Samples characterising the percentage contribution of the leaves of particular tree species to the litter. These samples were not precisely of a quantitative nature: from a surface area of 0.15–0.30 m^2 all the litter accumulated there was collected and placed in plastics bags. Each time 15 samples were collected. The aim was to obtain appropriately large representative material to be used for chemical analyses and accurate determination of the weight contribution of the individual plant species to the litter. The number of leaves in the material thus collected varied from several hundred to several thousand.

In the laboratory, the material was washed thoroughly in vibrating water, using a specially designed vibrator. Subsequently, after their separation from other litter fractions, the leaves were sorted according to whether they were of the 1973, or of the 1974 litter fall cycle. In addition to this, in the material derived from the two types of sample leaves of all the tree and shrub species occurring in the habitat under study were identified. From the leaves of each tree or shrub species discs of the size of 1.65 cm^2 were cut out at random (from areas not damaged by animals). As the fragmentation of leaves advanced the size of the discs was gradually reduced to 0.40 cm^2, or even 0.147 cm^2 during the final stages of leaf decomposition, and at the same time the number of discs in a series was increased: from 60 in the case of discs of the size 1.65 cm^2, up to 100, or even 200 – in the case of the 1.147 cm^2 discs. This precedure ensured a higher representativeness of the samples.

The samples were dried at about 60°C to a constant weight, and weighed to the nearest 0.01 mg.

The next stage in the investigation was the determination of chemical composition of the leaves. Leaves of each species were analysed for the so-called elementary composition: C, H, N, O and P. The C, H. N analyses were performed on a Carlo Erba mod. 1102 gas chromatograph. Each analysis was repeated 3–5 times. Oxygen content was determined indirectly on the basis of the known percentage of ash parts, and C, H, N according to the formula:

$$\%O_2 = 100 - (\%C + \%N + \%H + \%A) \tag{6}$$

where: A denotes the amount of ash parts.

The amount of ash parts was determined by calcining samples in the muffle furnace at 450°C. Phosphorus was determined by phospho-molybdate colorimetry. From the known elementary composition (C, H, N, O) the caloricity of a sample was calculated by using the formula suggested by Boie (1953) (after Krevelen and Schuyer 1959):

$$Q = 84.0\,C + 15\,N + 277.65\,H - 25.0\,S - 26.5\,O_2 \tag{7}$$

As the content of sulphur in plants is very low, being of the order of decimal percentages (S t a l f e s t 1972), this element was not taken into account.

4. RESULTS

In an alder wood, litter fall as a rule consists of leaves of one species, alder leaves representing about 97% of the total weight of all fallen leaves. Therefore in order to simplify the calculations presented it was assumed that the regularities found for the decomposition of alder leaves are representative for all the litter in this ecosystem.

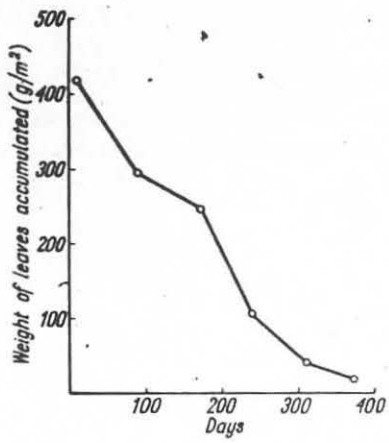

Fig. 1. Variations in the weight of leaves accumulated over 1 m^2 in a yearly cycle

In 1973, litter fall in the alder wood was complete by October 31, this point of time being considered to be the beginning of litter decomposition. Immediately after the ending of leaf fall the amount of litter accumulated on 1 m^2 surface was about 412 g. As assessed at the end of the growing season of the following year, the original quantity of 412 g/m^2 appeared to have been reduced down to as little as about 20 g/m^2 (Table I, Fig. 1). Microorganisms and saprophages were thus found to have consumed 95% of the litter during the yearly cycle, this indicating a rapid decomposition rate in the ecosystem studied.

Two phases can be noticed in the variation of the weight of 1 cm^2 of alder leaves. A fast initial decrease in the weight of 1 cm^2 leaf surface is followed by an increase of the weight, the rate of growth not being so high as the rate of decrease (Fig. 2, Table I). This growth is not accidental, and it has been found to be associated with the chemical changes of litter humification, where due to the addition of atmospheric oxygen molecules the weight of 1 cm^2 leaf surface increases[2]. Nevertheless, the changes in the weight of 1 cm^2 alder leaf surface could not be used for the calculation of microorganism consumption, because the quantity Δwg would in the particular case have had negative values. For this reason the commonly used method of expressing consumption in terms of calories was employed.

[2] This phenomenon will be the subject of a detailed study concerned with the methods of studying litter humification (in preparation).

Table I. Stages in the evaluation of litter consumption

Period of sample collecting	Time in days (t)	Weight of leaves accumulated over 1 m^2 (W) g/m^2	Weight of 1 cm^2 alder leaves (w) mg/cm^2	Surface of leaves accumulated over 1 m^2 (S) cm^2/m^2	Difference in leaf surface in t_0 and t_{+1} (S) cm^2/m^2	Average surface of leaves in t_0 and t_{+1} ($\bar{S}$) cm^2/m^2	Caloricity of 1 cm^2 alder leaves (w_{cal}) cal/cm^2
12 Nov. 1973	12	411.78	5.89	69,911.7			28,1*
29 Jan. 1974	90	297.96	4.51	66,066.5	3,845.2	67,989.1	22.9
23 April 1974	175	247.76	4.39	56,437.3	9,629.2	61,251.9	21.2
25 June 1974	238	108.78	4.59	23,699.3	32,738.0	40,068.3	20.5
6 Sept. 1974	311	41.20	4.71	8,747.3	14,952.0	16,223.3	19.9
6 Nov. 1974	372	19.66	4.54	4,330.4	4,417.0	6,538.9	19.5

*Data obtained by approximating empirical results to the exponential function.

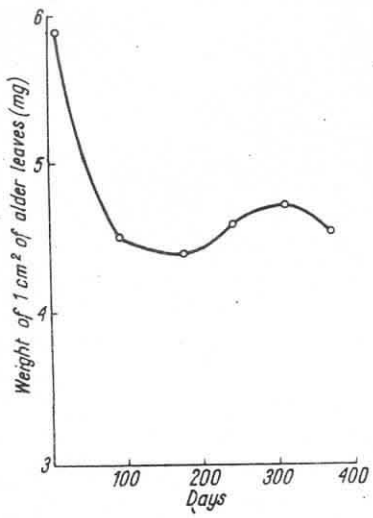

Fig. 2. Variation of the weight of 1 cm^2 alder leaves over a yearly cycle

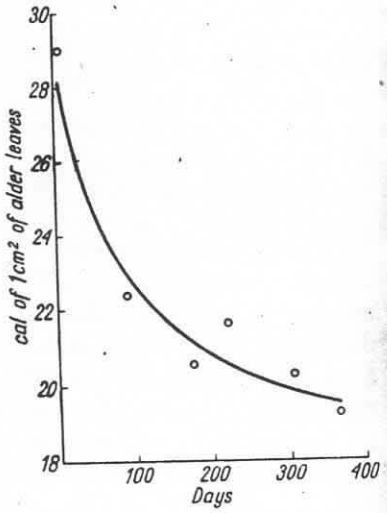

Fig. 3. Variation of the caloricity of 1 cm^2 alder leaves over a yearly cycle

Changes in the caloricity of 1 cm^2 alder leaves in consecutive points of time are indicated by a steady decrease, at first rapid and then slower and slower (Fig. 3). The changes presented may be adequately described by the exponential function $y = 37.90x^{-0.1124}$ where: y is the caloricity of 1 cm^2 alder leaves, x – decomposition time expressed in days. The correlation coefficient of this relation is high, being equal to $r = 0.96$, the significance being at the level of < 0.01.

in the alder wood by microorganisms and saprophages

Difference in the caloricity of 1 cm² alder leaves in t_0 and t_{+1} (Δw_{cal}) cal/cm²	Average caloricity of 1 cm² alder leaves in t_0 and t_{+1} ($\bar{w}_{cal}$) cal/cm²	Microorganism consumption (C_M) kcal/m²	Saprophage consumption (C_S) kcal/m²	Total consumption (ΣC) kcal/m²	Microorganism consumption per day kcal/m²/day	Saprophage consumption per day kcal/m²/day
5.20	25.50	353.54	98.05	451.6	4.53	1.26
1.70	22.05	104.13	212.32	316.4	1.22	2.50
0.70	20.85	28.05	682.59	710.6	0.44	10.83
0.60	20.20	9.73	302.03	311.8	0.13	4.14
0.40	19.70	2.61	87.01	89.6	0.04	1.43
	Σ kcal/m²/year	498.1	1,382.0	1,880.1		

Stages in the calculation of microorganism and saprophage consumption are shown in Table I. Given the weight of leaves per 1 m², and the weight of 1 cm² of leaves, the total surface of leaves accumulated (S) could be calculated for each consecutive point of time, and then ΔS and $\bar{S}$. The caloricity of 1 cm² was in turn used for the calculation of Δw_{cal} and $\bar{w}_{cal}$. The last stage was the calculation of saprophage and microorganism consumption according to formulae (3) and (5).

Total annual litter losses in an alder wood amount to 1,880 kcal/m². The share of microorganisms in this total is 498 kcal/m²/year, saprophage consumption being considerably higher – 1,382 kcal/m²/year (Table I). It is therefore mainly the saprophages that are responsible for the overall, very fast, rate of litter disappearance from the ecosystem under study – 73.5% of the annual loss, as opposed to 26.5% of losses due to the activity of the microorganisms.

It is worth noting the yearly variation in the rate of litter consumption by the destruent groups considered. For microorganisms the highest litter consumption rate was recorded for the period following leaf fall, autumn and winter, after which a steady drop of the rate was seen (Fig. 4). Thus the activity of the microorganisms does not follow the temperature standard, for it is during the summer and spring months that the temperature peaks occur. It seems that the main factor limiting the activity of microorganisms may be the amount of available organic substances in the litter. Alder leaves are very rich in organic nitrogen, forming about 2.9–3.0% of their dry weight. The presence of large quantities of building material available to the microorganisms may result at first in their rapid multiplication, and consequently exhaustion of the supplies of these substances. Such a hypothesis would be supported by the observations published by Robinson and McDonald (1964), Minderman and Daniels (1967), and by Nicholson, Bocock and Heal (1967). Minderman and Daniels (1967) have found that the falling litter is almost immediately colonised by microorganisms (within several days). Following the autumn peak abundance of bacteria, their numbers gradually decrease, which would agree with the microorganism activity described. A similar microorganism activity peak, occurring during early stages of decomposition, as in the present study, was observed by Nicholson, Bocock and Heal (1967) who

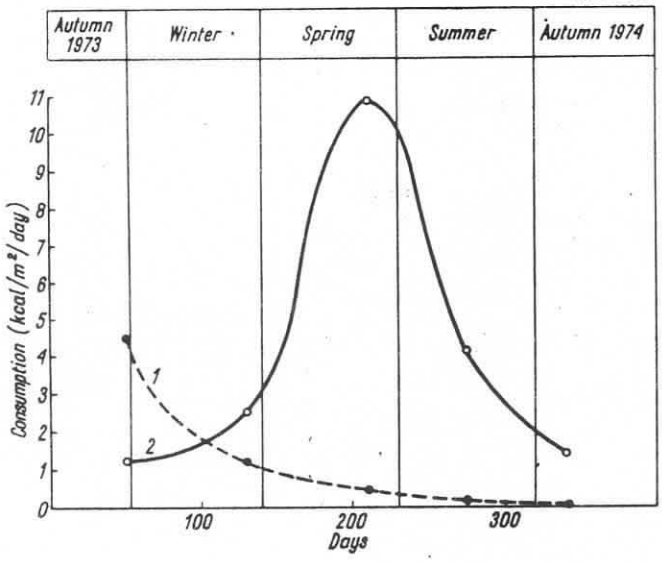

Fig. 4. Microorganism and saprophage consumption over a yearly cycle
1 – microorganisms, *2* – saprophages

investigated the decomposition of the faeces of *Glomeris marginata* Villers. On the other hand, an addition of readily assimilable substances to the soil results in a considerable increase of microorganism activity (R o b i n s o n and M c D o n a l d 1964). Of course, this is only one of the possible hypotheses to explain the picture of the variations in microorganism activity, observed during the study, which may also depend on the overcrowding of microorganisms caused by a rapid multiplication, or some chemical changes associated with litter humification.

The annual course of saprophage activity is quite different. During the period immediately following the foliage fall, they eat relatively small amounts of litter. Their activity reaches its peak during spring, whereafter a decrease of their consumption is observed (Fig. 4). By contrast to the microorganisms, this is a temperature activity model, similar to the annual distribution of temperature. The characteristic feature of saprophage activity is the immediateness of their action, not preceded by any initial phase of microbiological transformation of the litter. This may be attributed to the initial content of nitrogen compounds in the alder leaves. The subsequent microbiological "processing" of such a litter contributes only a small extent to the lowering of the C/N ratio. On account of the high content of nitrogen alder leaves are, therefore, as it were, potentially predestined to an immediate consumption by saprophages.

5. DISCUSSION

One of the most serious objectives raised to the techniques used for studying litter decomposition processes is the inadequate nature of the results, relative to the natural conditions, caused by the experimental procedure employed by the investigator. For instance, as stressed by W i t k a m p and O l s o n (1963), leaves decaying in bags are usually moister than they would be in natural conditions, and as a result there occurs a change in the intensity of their

decomposition. Objections of this kind do not, of course, apply to the method presented in this paper, because it does not include any handling that might disturb the natural course of the decomposition processes, or alter the activity of one of the destruent groups. In spite of its unquestionable advantages there is a feature in this method which may to be disadvantageous, namely the lack of clear-cut criteria for the assessment of the activity of each of the groups considered: microorganisms and saprophages. If, for instance, some saprophage groups ate litter without any visible damage to the leaves, causing only, like the microorganisms, their "thinning", the method applied would include their activity in the activity of the microorganisms. Consequently, the microorganism consumption of the litter would become artificially increased, whereas that of the saprophages – artificially lowered. In order to check whether this is possible, a comparison was made of the annual course of changes in the content of phosphorus and carbon per 1 cm^2 alder leaves. It was found that the level of P per 1 cm^2 remained unchanged throughout the year, while the level of carbon per 1 cm^2 steadily decreased (Fig. 5). If the level of elements per 1 cm^2 were to any extent affected by the saprophages, then, on account of their specific feeding – gnawing the leaves – we should observe a parallel decrease of the content of carbon and phosphorus, but since this is not the case, the level of phosphorus remaining unchanged, the conclusion is that the method used precisely separates the consumption of either of the destruent groups considered.

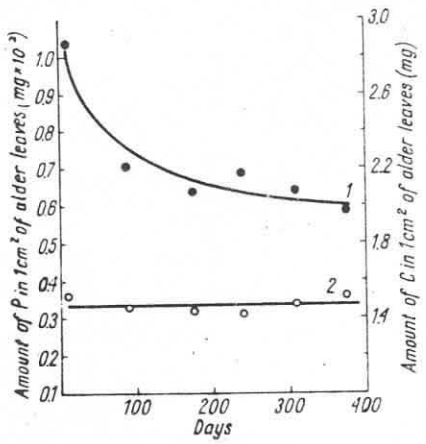

Fig. 5. Variations in the content of carbon and phosphorus in 1 cm^2 alder leaves over a yearly cycle
1 – carbon, *2* – phosphorus

It seems, therefore, that the procedure presented by the authors may provide a useful method of analysing the litter decomposition processes under fully natural conditions without any experimental interference from the investigator, and, what is even more important, without using any techniques based on the application of radioactive elements.

6. SUMMARY

The aim of the present paper is to describe methods of evaluating saprophage and microorganism consumption in the litter, without any experimental interference from the investigator. The methods concerned are based on two assumptions: saprophage consumption may be considered as a loss in

leaf-surface, and the microorganism consumption – as losses in leaf-thickness, which is manifested by a changed weight of 1 cm^2 leaf surface. This is expressed by the formulae (3) and (5).

Using the formulae the authors have carried out example calculations for an alder wood (*Carici elongatae-Alnetum*). It has been found that out of the total annual loss, amounting to 1,880 kcal per 1 m^2, 26.5% (498) is due to microorganism consumption, and 73.5% (1,382) – due to saprophage consumption (Table I). The highest rate of leaf consumption by microorganisms was recorded during the period following the autumn foliage-fall, and by saprophages – during the spring-summer months (Fig. 4). The authors have put forward a supposition that in the case of saprophages the factor responsible for the rate of litter consumption is the temperature, and in the case of microorganisms – the amount of readily assimilable organic substances.

The method presented makes it possible also to separate the consumption of each destruent group. The fact that the level of phosphorus per 1 cm^2 alder leaf surface, as found during the present study, remained unchanged throughout the period considered (Fig. 5) indicates that in this method consumption caused by saprophages is not included in that occurring due to the activity of microorganisms. If saprophages could affect the weight of 1 cm^2 leaf surface, this level would not remain unchanged, but would instead decrease gradually.

7. POLISH SUMMARY (STRESZCZENIE)

Celem pracy było opisanie metod oceniających konsumpcję saprofagów i mikroorganizmów w ściółce bez jakiejkolwiek eksperymentalnej ingerencji badacza. Metodyka oparta została na dwu założeniach: konsumpcję saprofagów można sprowadzić do ubytków powierzchni liści, konsumpcję spowodowaną przez mikroorganizmy – do ubytków grubości liścia, co wyraża się zmianą ciężaru 1 cm^2 liścia. Ujmują to wzory (3) i (5).

Opierając się na powyższych wzorach dokonano przykładowych obliczeń dla olsu (*Carici elongatae-Alnetum*). Stwierdzono, że z 1880 kcal ubywających rocznie z m^2 26,5% (498) jest wynikiem konsumpcji mikroorganizmów, 73,5% (1382) rezultatem konsumpcji saprofagów (tab. I). U mikroorganizmów najszybsze tempo konsumowania liści przypada na okres po jesiennym opadzie listowia, u saprofagów – na miesiące wiosenno-letnie (fig. 4). Autorzy wysunęli przypuszczenie, że u saprofagów czynnikiem odpowiedzialnym za tempo konsumowania ściółki jest temperatura, u mikroorganizmów zaś – ilość łatwo przyswajalnych substancji organicznych.

Opisana metodyka pozwala również dokładnie rozgraniczyć konsumpcję obu grup destruentów. Konsumpcja spowodowana przez saprofagi nie może być zaliczana jako konsumpcja wynikająca z oddziaływania mikroorganizmów, gdyż ilość fosforu na 1 cm^2 liścia olchy utrzymuje się na stałym poziomie przez cały okres (fig. 5). Gdyby na ciężar 1 cm^2 liścia mogły mieć wpływ saprofagi, poziom ten nie mógłby osiągnąć stałych wartości; musiałby sukcesywnie obniżać się.

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